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Epidermal Cell Fate and Patterning in Leaves

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INTRODUCTION

Cell differentiation requires that undifferentiated cells first be selected before becoming committed to a specific fate. The selection of precursor cells often is coordinated so that mature differentiated cells are distributed in a characteristic pattern. One of the simplest possible patterns in tissues is that in which a minimum distance is maintained between differentiated cells in a two-dimensional sheet of cells (Wolpert, 1971). Such a pattern could be created by several different mechanisms. For example, the initial positioning of precursor cells could be random within a field of equally competent cells, with adjacent cells subsequently prevented from assuming the precursor cell fate by lateral inhibition. Alternatively, a prepatterning could exist so that the selection or placement of the precursor cells is nonrandom. Regardless of how precursor cells are placed, the production of new cells from a precursor cell can also contribute to the final spacing pattern (Sachs, 1978). Although the molecular interactions guiding patterning are known for such model systems as epidermal bristle formation in Drosophila (Ghysen et al., 1993), little is known about the nature of the intercellular signaling that establishes cell patterning in plants (see Clark, 1997; Kerstetter and Hake, 1997; Laux and Jürgens, 1997; McLean et al., 1997; Poethig, 1997; and Schiefelbein et al., 1997, in this issue, for further discussion).

The epidermis of plant leaves provides an excellent system for analyzing pattern formation because the epidermal surface is readily accessible and cell patterns can be analyzed within a plane rather than in three dimensions. The leaves of most plants contain two highly differentiated cell types in the epidermis: guard cells, which constitute stomata, and trichomes. These cells, the spacing of which is the primary focus of this review, are usually separated from each other by pavement cells. Figure 1 illustrates these three classes of cells in an Arabidopsis leaf.

The same two questions can be asked regarding the developmental patterns of trichomes and stomata: How are the precursor cells selected, and how is the spacing pattern generated? However, the answers appear to be quite different for each cell type. Although patterning in Arabidopsis is considered in the greatest detail in this article, data from other species also are discussed.

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TRICHOMES

Trichomes are found on the aerial epidermal surfaces of almost all major groups of terrestrial plants. Morphologically, trichome structure is diverse (Theobald et al., 1979). On some plants, a single trichome type is found, whereas on many other species, several different types are found (Esau, 1977). For example, six different types of trichomes are found on *Lycopersicon esculentum*. These range from large multicellular spikes to small trichomes containing several gland cells that are perched atop a two-celled stalk (Reeves, 1977). The unicellular trichomes on Arabidopsis leaves most often consist of a stalk and two to four branches (Figure 1A). The timing of trichome development also varies. In Arabidopsis and many other species, trichomes are the first cells that terminally differentiate on young leaf primordia (Larkin et al., 1996). By contrast, the trichomes that develop on the sepals of *Salvia splendens* and on the ovules of *Gossypium hirsutum* (the latter of which ultimately form the cotton fibers) initiate only after other epidermal cells have stopped dividing (Korn, 1994).

Trichome Spacing

By using a measure of spatial distribution first applied in ecological studies (Clark and Evans, 1954), a minimum distance spacing pattern of trichomes has been demonstrated for several plants, including Arabidopsis (Korn, 1994; Larkin et al., 1996). This nonrandom distribution could result from one of two different mechanisms. The first model posits an inhibitory signal that prevents neighbors from taking the same developmental fate (Bünning and Sagromsky, 1948; Lawrence and Hayward, 1971; Wolpert, 1971; Wolpert and Stein, 1984; Korn, 1994; Larkin et al., 1996). The second is a cell lineage model that proposes that after a precursor cell is

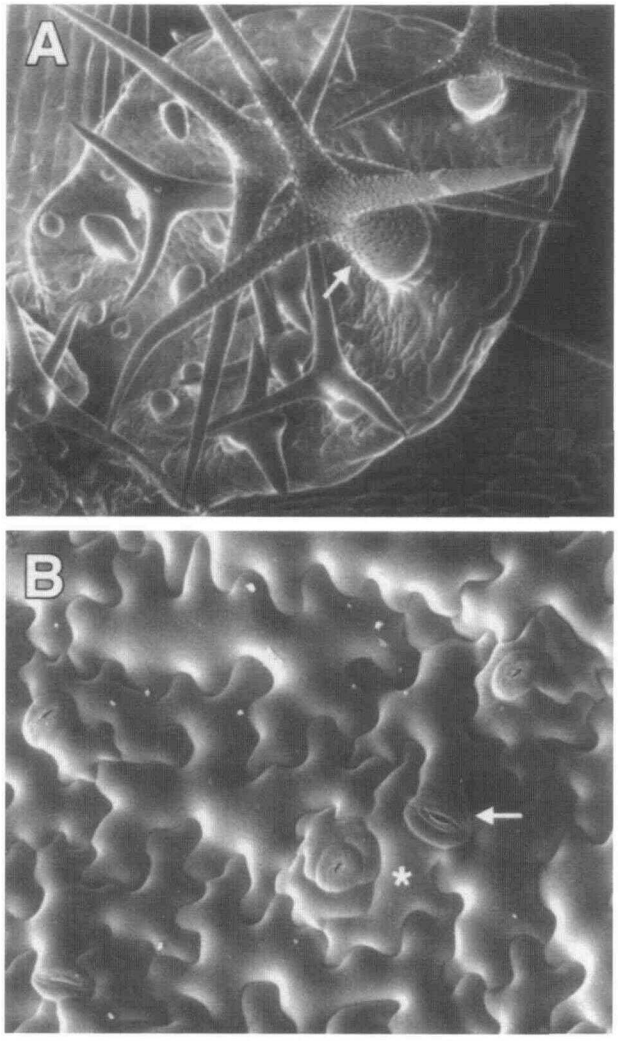

Figure 1. Cell Types in the Epidermis of Arabidopsis Leaves and Cotyledons.

(A) Scanning electron micrograph of an expanding leaf, showing mature (arrow) and developing trichomes.
(B) Cryo-scanning electron micrograph of the abaxial epidermis of a cotyledon. Stomata (arrow) are separated from each other by intervening pavement cells (*).

selected, it undergoes an intrinsic pattern of cell division that separates the final differentiated structure from its neighbors (Sachs, 1978, 1996). These two mechanisms are not mutually exclusive because spacing could be controlled by a combination of programmed cell division patterns and cell signaling (see Stomatal Patterning, below).

The lineage model predicts that the differentiated structure will be surrounded by its immediate clonal siblings. This hypothesis has been tested by using clonal analysis in Arabidopsis (Larkin et al., 1996). Plants containing a cauliflower mosaic virus *35S* promoter–β-glucuronidase (GUS) reporter gene that had been inactivated by the insertion of the maize *Activator* (*Ac*) transposon formed the basis for these analyses. *Ac* transposition during early plant development resulted in large sectors of clonally related GUS-positive cells in these plants (Lawson et al., 1994). The boundaries of GUS-positive sectors in these plants were found to pass through trichomes and adjacent cells at random, which is inconsistent with a major role for cell lineage in generating the spacing pattern (Larkin et al., 1996). Instead, these results suggest that a model based on inhibitory interactions between cells may explain more effectively the observed trichome spacing pattern.

Because any individual sector boundary that passes between a trichome and its neighbors can eliminate only a subset of the neighbors from the trichome lineage, it has been suggested that cell lineage programs could still play a role in patterning (Sachs, 1996). For lineages that do not completely surround each trichome to play a role in the spacing pattern, these lineages would need to be regularly oriented relative to the axis of the leaf. The lineages observed in the clonal analyses were highly variable and thus difficult to reconcile with a trichome lineage unit that has a consistent polarity relative to the leaf axis. Nevertheless, it is still possible that such lineages exist but are modified by cell–cell interactions that correct the pattern, as proposed by Sachs (1996). To resolve this issue, it would be useful to observe cell lineages associated with trichome development directly in living tissue by using the dental impression technique (Williams and Green, 1988).

Role of Asymmetric Cell Divisions

Some earlier work suggests that many cellular differentiation events require an asymmetric division of a mother cell into two unequal daughters, which go on to assume different developmental fates. In grasses, trichomes clearly originate from an asymmetric division (Esau, 1977). In species of the monocot genus *Anemarrhena*, the files of cells that give rise to trichomes appear to originate from a division that is longitudinal but oblique to the paradermal plane (Rasmussen, 1981). In *Peperomia*, glandular trichomes sometimes arise from an unequal division, although this is not always the case. Moreover, the division plane is usually different from that used for the initiation of the stomatal meristemoid (see below; Sachs and Novoplansky, 1993). Similarly, Esau (1977) presented a description of trichome development in *Ligustrum* that gave no indication of an asymmetric division. Thus, although asymmetric divisions do give rise to trichome initiation in some plants, they are by no means universal.

Careful examination of developing leaves by light and scanning electron microscopy failed to reveal any evidence for an asymmetric division involved in Arabidopsis trichome initiation (J.C. Larkin and M.D. Marks, unpublished data). In addition, trichome development can be induced artificially

without an intervening cell division (Lloyd et al., 1994), indicating that an asymmetric cell division is not required for trichome initiation.

GENETIC AND MOLECULAR ANALYSES OF TRICHOME DEVELOPMENT

Mutations affecting trichome initiation and density have been described in several plant species (Reeves, 1977; Lee, 1985; Bowley and Lackle, 1989; Goffreda et al., 1990; Kloth, 1993, 1995; Hombergen and Bachman, 1995). However, the most extensive genetic analyses have been conducted on the initiation and spacing of Arabidopsis trichomes. These analyses have included the molecular characterization and manipulation of several genes involved in Arabidopsis trichome formation (recently reviewed in Marks, 1997).

GLABRA1 (*GL1*) and *TRANSPARENT TESTA GLABRA* (*TTG*)

Two genes, *GL1* and *TTG*, are required for the initiation of trichome development on most epidermal surfaces of Arabidopsis. Plants homozygous for strong recessive alleles of either gene are virtually devoid of trichomes. *gl1* mutants are defective only in trichome development, whereas *ttg* plants also lack anthocyanin and seed coat mucilage (Koornneef, 1981) and have extra root hairs (Galway et al., 1994). Mutations in *gl1* and *ttg* are epistatic to all other trichome mutations examined to date, with the possible exception of *reduced trichome number* (*rtn*; see below).

GL1 encodes a protein with sequence similarity to the DNA binding domain of the *MYB* family of transcriptional regulators (Oppenheimer et al., 1991). *GL1* transcripts, which are present at a low level throughout the leaf protoderm, accumulate to a high level in trichome precursors during early trichome development (Larkin et al., 1993).

Results from two different mosaic analyses suggest that the *GL1* gene acts through a cell autonomous mechanism. Rédei (1967) exposed seeds from a *GL1/gl1* heterozygous plant to x-rays and identified glabrous sectors on the resulting plants. More recently, heterozygous *gl1* mutant seeds were treated with ethyl methanesulfonate, and the resulting plants produced glabrous sectors that were not seen on wild-type plants (Hülskamp et al., 1994). The glabrous patches in both studies could result from the uncovering of the *gl1* mutant allele in heterozygous plants. Their presence indicates that cells outside the sector cannot provide a diffusible substance or signal to overcome the effect of the probable *gl1* mutation. These results, although not sufficient to demonstrate directly the cell autonomy of *GL1* action, are consistent with the primary function of *GL1* being confined to the trichome precursor cell.

TTG has not been isolated; however, expression of the maize *R* gene, which encodes a protein with sequence similarity to the helix-loop-helix family of transcriptional activators (Ludwig et al., 1989), in *ttg* mutant plants results in functional complementation of all aspects of the *ttg* mutation (Lloyd et al., 1992). This suggests but does not prove that *TTG* may encode a homolog of the maize *R* gene. In any case, the *R* gene has been a useful reagent that can provide ectopic *TTG* function.

Transgenic plants expressing *GL1* and *R* under altered regulatory control have been used to dissect trichome initiation. Transgenic plants that constitutively express both genes form numerous hairs on all shoot epidermal surfaces, including those upon which trichomes do not normally form (Larkin et al., 1994). Additionally, constitutive expression of *GL1* cannot bypass the need for TTG protein, and that of *R* cannot bypass the need for GL1. These results provide genetic evidence indicating that *GL1* and *TTG* may act at the same step in trichome development (Larkin et al., 1994). Furthermore, we have recently demonstrated that GL1 can form a complex with R (R. Jilk and M.D. Marks, unpublished results), suggesting that the GL1 and TTG proteins may interact physically.

To determine when *R* can function in Arabidopsis, Lloyd et al. (1994) generated a chimeric gene in which *R* was fused to the sequence encoding the steroid binding domain of the rat glucocorticoid receptor gene. It is thought that in the absence of steroid, the chimeric R–glucocorticoid fusion protein is sequestered in the cytoplasm; however, in the presence of steroid, it enters the nucleus. When this construct was expressed in *ttg* mutant plants, trichome formation could be induced by applying the glucocorticoid steroid analog dexamethasone. When trichome initiation was induced by hormone treatment relatively late in leaf development, the epidermis in distal (older) regions of the leaf could not be induced to form trichomes, indicating that once the epidermis reaches a certain developmental stage, it is no longer competent for trichome development.

RTN

RTN may be involved in controlling the duration of the phase during which leaves are competent to initiate trichomes (Larkin et al., 1996). This gene was identified as an allelic variant in two commonly used wild-type Arabidopsis strains, Landsberg *erecta* (L*er*) and Columbia (Col). Col plants have ~30 trichomes on the first leaf, whereas L*er* plants usually have <12. It was possible to map this trait to chromosome 2 by using a qualitative trait locus analysis of the Lister and Dean recombinant inbred lines, which were generated using Col and L*er* (Lister and Dean, 1993; Larkin et al., 1996). It was shown that the duration of trichome initiation in plants bearing the L*er* allele is shorter than that of plants carrying the Col allele. It is therefore possible that *RTN* may control

either the timing of *TTG* or *GL1* expression or the time during which epidermal cells can respond to *GL1* and *TTG*.

Genes Involved in Spacing

The above analyses indicate that genes such as *GL1* and *TTG* are involved in the process through which cells become trichomes, but they say little about whether these genes also participate in the control of trichome spacing. However, two lines of evidence suggest that both *GL1* and *TTG* may control a pathway that prevents epidermal cells from assuming the trichome cell fate. First, weak mutant alleles of both *GL1* and *TTG* have been identified that do not completely suppress trichome initiation; the trichomes that develop on these mutants often are found in clusters (Esch et al., 1994; Larkin et al., 1994). Second, heterozygous *TTG/ttg* plants that ectopically express *GL1* (and are likely to overproduce GL1 protein) produce numerous trichome clusters (Larkin et al., 1994). This latter result suggests that a stoichiometric balance between the relative concentrations of GL1 and TTG protein may be required to prevent clustering. *TRYPTYCHON* (*TRY*) has been identified as a gene that could act downstream of *GL1* and *TTG* in the proposed inhibitory pathway (Hülskamp et al., 1994). Mutations in this gene increase the number of trichome clusters.

MODEL FOR LEAF TRICHOME INITIATION AND SPACING IN ARABIDOPSIS

Taken together, these results suggest the following model, which is also presented in Figure 2. All protodermal cells are assumed to have an equivalent potential to develop as trichomes. The GL1 and TTG polypeptides are proposed to act together as a heterodimeric transcriptional activator that promotes trichome initiation; commitment of a cell to the trichome differentiation pathway is presumed to be directly controlled by the level of the GL1/TTG heterodimer. In addition, *GL1* expression, and possibly *TTG* expression, is presumed to be controlled by a positive feedback loop either directly by autogenous regulation or indirectly by the action of downstream genes. This loop would explain the rapid increase in *GL1* expression that is seen concomitant with trichome initiation. Activation of this pathway also results in the production of an inhibitory signal, mediated by the *TRY* gene product, which inhibits neighboring cells from differentiating as trichomes.

In competent protodermal tissue, neighboring cells initially are locked in a mutual inhibition, and all cells continue dividing (Figure 2A). Because of stochastic fluctuations, individual cells eventually overcome this inhibition. GL1/TTG levels begin to rise in those cells, which stop dividing and begin to differentiate. The inhibitory signal from these differentiating cells, termed precursor cells, strengthens, thereby preventing their immediate neighbors from becoming trichomes (Figure 2B). Positive feedback loops controlling GL1/TTG levels in adjacent cells are coupled by negative feedback loops acting between cells, and adjacent cells are forced to assume different fates. Competence of the tissue to form trichomes can be viewed as a metastable intermediate state that leads to the trichome cell fate.

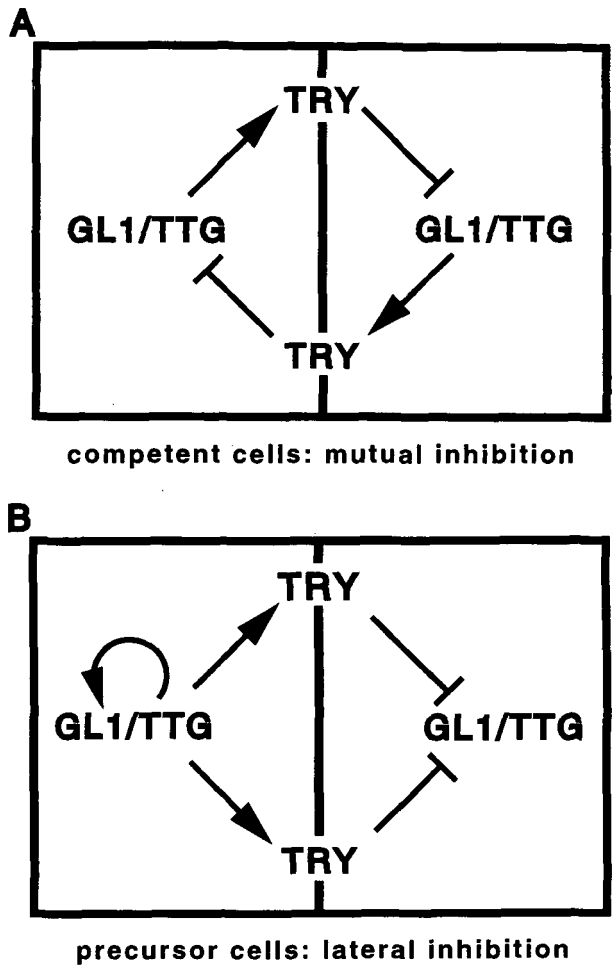

Figure 2. Genetic Model for the Selection of Trichome Precursor Cells.

(A) Mutual inhibition of trichome formation. Two competent protodermal cells are locked in a metastable state of mutual inhibition.
(B) Lateral inhibition of trichome formation. A trichome precursor cell (left) inhibiting its neighbor (right) from becoming a trichome.
A similar model has been proposed for the spacing of bristles in Drosophila (Ghysen et al., 1993). Arrows, positive regulatory interactions; T-bars, negative regulatory interactions.

Although this model is consistent with the available facts, much work remains to be done before it can be considered more than speculative. Of paramount importance are the cloning and characterization of the *TTG* gene. Where and

when is *TTG* expressed? Does the protein interact with GL1 TTG in the same manner as it does with R? It will also be important to characterize further the expression of *GL1*. How is the basal pattern of *GL1* expression established? What is the mechanism by which *GL1* is upregulated upon determination of a cell as a trichome precursor? Does this process involve an inhibitor, or is inhibition mediated by direct cellular contacts? What role does the *TRY* gene play in this process? The answers to these and other questions will provide a firmer basis for understanding how trichome precursor cells are selected and how a simple developmental pattern is created.

STOMATAL PATTERNING

Stomata consist of two guard cells that surround a pore connecting the atmosphere to the internal air spaces of the leaf (Sack, 1987; Willmer and Fricker, 1996). Unlike trichomes, stomata are essential for plant survival, and the evolution of the genes necessary for the creation of stomata was a key event in the emergence of land plants. The patterning of stomata has received much attention (reviewed in Sylvester et al., 1996) and is reviewed here for both monocots and dicots, although Arabidopsis is emphasized.

Nature of Patterning

A major and universal feature of stomatal patterning is the presence of a stomate-free region around each stomate (Figure 1B). Indeed, the frequency of stomata that contact other stomata is much lower than would be found in a random distribution (Sachs, 1978, 1991; Korn, 1992). Instead, stomata are separated by a minimum distance of at least one intervening epidermal cell so that a stomatal spacing pattern is established. The adaptive advantages of this pattern may be that it minimizes overlap between gaseous diffusion shells, ensures the presence of epidermal cells adjacent to stomata (so-called neighbor cells) as sources of ions, and establishes efficient ratios between the pore area and the photosynthetic capacity of underlying mesophyll cells.

Another aspect of patterning is that stomata are usually absent over veins, and in some plants stomata do not form over specific cell types in the mesophyll (Rasmussen, 1986; Smith and Watt, 1986; Croxdale et al., 1992). This suggests either that the distribution of internal cells affects the placement of stomata or that both anatomy and stomatal distribution are patterned coordinately. The distribution of mature stomata has been described as random in dicots (Sachs, 1978), except for the placement of stomata with respect to internal features and the absence of stomata in contact. The latter is emphasized here because little is known about the role of anatomy in stomatal patterning and because of the isolation of mutants that violate the minimum spacing rule.

Several different developmental mechanisms seem to operate to establish the lateral stomatal spacing pattern. Key elements include the frequency and placement of asymmetric divisions that produce stomatal initials, the production of both ordinary epidermal cells and guard cells by the stomatal initial, the importance of cell–cell communication in orienting divisions and in regulating precursor cell activity, and the role of developmental timing in controlling when cells are competent to form stomatal initials and where those initials are formed. Underlying these mechanisms is the more fundamental question of the relative contributions of cell position and cell lineage to determining the choice of plant cell fate (see Clark, 1997; Kerstetter and Hake, 1997; Laux and Jürgens, 1997; McLean et al., 1997; Poethig, 1997; and Schiefelbein et al., 1997, in this issue, for additional discussion).

Stomatal Initials Are Produced in an Asymmetric Cell Division

In both monocots and dicots, stomata develop as a result of cell divisions that are asymmetric in both their geometry and the fate of the resulting daughter cells (Rasmussen, 1981). These divisions are usually highly polarized in the placement of the premitotic nucleus and in the distribution of cytoplasm (Galatis and Mitrakos, 1979).

In most monocots, the smaller of the two cells produced by an asymmetric division is usually rectangular. This cell becomes a guard mother cell and divides symmetrically to produce two guard cells, as illustrated in Figure 3A (Tomlinson, 1974). In most dicotyledons, the smaller cell, which usually is triangular, divides several times before converting into a guard mother cell, as illustrated in Figure 3B (Galatis and Mitrakos, 1979). Bünning (1953) termed dicot stomatal initials meristemoids to emphasize that they continue to divide after the surrounding cells have stopped dividing. Primary meristemoids are found in all dicots and are formed after the asymmetric division of a protodermal cell, which functions as a meristemoid mother cell. Some dicots, such as Arabidopsis, have an additional type of meristemoid, a satellite meristemoid (termed a secondary meristemoid in Landré, 1972), which is produced by the asymmetric division of a neighbor cell (Figure 3C). Thus, there are at least three types of stomatal initials in angiosperms, primary and satellite meristemoids in dicots and rectangular initials (termed short cells in grasses) in most monocots. As discussed below, how the stomatal pattern is generated depends on the type of initial.

Generation of Stomatal Pattern by Placement of the First Asymmetric Division

Whereas primary meristemoids seem to be positioned randomly, the placement of monocot initials and satellite meristemoids is highly regulated and is crucial for stomatal patterning.

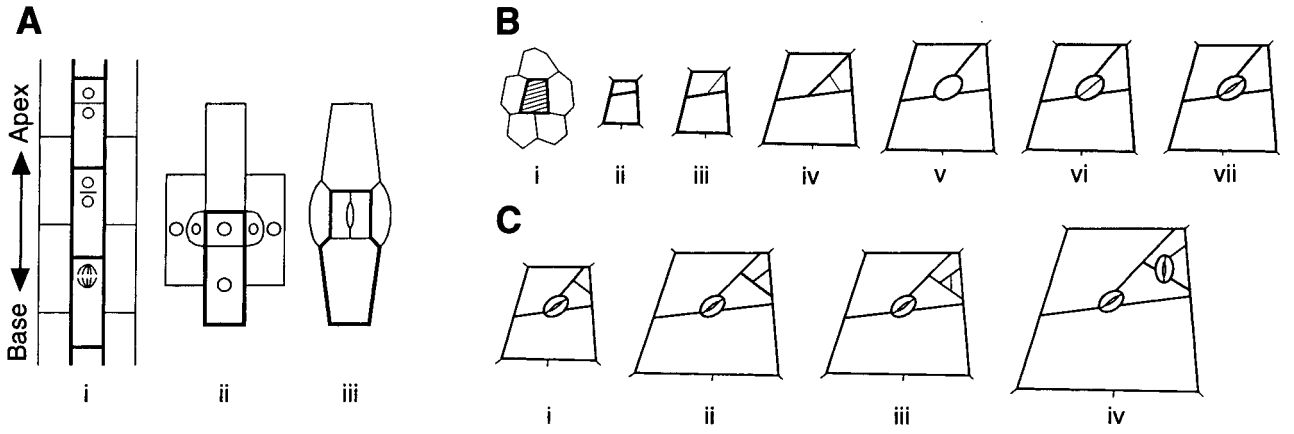

Figure 3. Stomatal Initials and Patterning in Grasses and Arabidopsis.

(A) Stomatal patterning in grasses. (i) Each stomatal initial forms closer to the leaf apex. New transverse walls are offset from walls in adjacent files. (ii) Subsidiary cells form in asymmetric divisions in adjacent cell files. (iii) The guard mother cell divides symmetrically to form two guard cells that surround a pore. The lower neighbor cell originated in the asymmetric division in (i); the darker line outlines the two cells produced by that division.

(B) Formation of a stomatal complex from a primary meristemoid in Arabidopsis. The oriented production of neighbor cells can create stomatal patterning, regardless of the original placement of the primary meristemoid. (i and ii) A primary meristemoid forms through the asymmetric division of a protodermal cell (shaded) that functions as a meristemoid mother cell. (iii and iv) Additional asymmetric divisions are oriented so that the primary meristemoid becomes placed approximately in the center of the future stomatal complex. (v) The triangular meristemoid converts to an oval guard mother cell. (vi and vii) The guard mother cell divides symmetrically to form two guard cells, which then differentiate.

(C) Formation of a stomatal complex from a satellite meristemoid in Arabidopsis. The initial placement of the satellite meristemoid establishes stomatal patterning regardless of the number of subsequent oriented divisions. (i) Satellite meristemoids form in an asymmetric division of a neighbor cell. The placement of this type of meristemoid is regulated so that it forms away from an existing stomate. (ii and iii) Satellite meristemoids can also divide asymmetrically to produce additional neighbor cells (iv).

In monocots, the polarity of asymmetric divisions is coordinated so that the initial forms toward the leaf tip and the larger cell product is closer to the leaf base, resulting in the alternation of initials and future neighbor cells (Figure 3A; Tomlinson, 1974; Rasmussen, 1986). This coordination of polarity is essential for patterning because the short cell develops directly into a guard mother cell without further divisions.

In dicots, the polarity of divisions leading to the formation of satellite meristemoids also appears to be tightly regulated. The formative asymmetric division in the neighbor cell is oriented so that the smaller daughter cell, the satellite meristemoid, becomes located away from the existing stomate and the larger daughter cell becomes a new intervening neighbor cell (Figure 3C; Landré, 1972; Galatis and Mitrakos, 1979). The satellite meristemoid can then convert directly into a guard mother cell. In this case, the original placement of the satellite meristemoid is critical to prevent the formation of stomata in contact. Satellite meristemoids also often divide asymmetrically one or more times through a series of oriented cell divisions, thus producing additional neighbor cells that further separate the two stomata (Figure 5A).

By contrast, the placement of the division that produces primary meristemoids is thought to be generally random in dicots (for exceptions, see Payne, 1979; Pappas et al., 1988). Moreover, so far as is known, the selection of the protodermal cells that are to become meristemoid mother cells also appears to be random (Sachs, 1994). Thus, patterning of stomata derived from primary meristemoids is generated by oriented divisions of the meristemoids (Figure 3B; Sachs, 1978). Although subsequent asymmetric divisions of the primary meristemoid are spatially regulated, the first division that creates the meristemoid usually is not.

Generation of Stomatal Pattern by Oriented Divisions of the Meristemoid

The production of neighbor cells by a meristemoid can in itself create a stomatal spacing pattern. In Arabidopsis, many meristemoids produce two neighbor cells sequentially so that the guard mother cell is positioned toward the center of the stomatal complex (Figure 3B, diagrams ii to v; Paliwal, 1967). This series of oriented asymmetric cell divisions creates a stomate-free region consisting of neighbor cells that

are derived from the same meristemoid mother cell as the stomate itself (including the neighbor cell that develops from the larger product of the first asymmetric division; Figure 3B, diagram ii). This cell lineage–based method of stomatal patterning involves both the production and oriented placement of neighbor cells from a single meristemoid. Regardless of the initial placement of the primary meristemoids, a cell lineage mechanism can create stomatal patterning so long as a full complement of neighbor cells is produced and each asymmetric division is properly oriented.

Stomatal patterning can also be created by the spatial coordination of the divisions of cells derived from two primary meristemoids. For example, it has been observed that when two primary meristemoids form in contact, the next asymmetric divisions are positioned such that the smaller daughter cells (regenerated meristemoids) become separated from each other (Kagan et al., 1992; Sachs, 1992). Such oriented divisions are especially important for patterning when primary meristemoids do not produce a full complement of neighbor cells. In these cases, one or more neighbor cells derive from a cell lineage that differs from the one that produced the stomate; here, the coordinated placement of neighbor cells across cell lineages ensures the absence of stomata in contact.

Stomatal Development Involves a Series of Cell Fate Choices

In dicots, several cell fate choices occur in the stomatal cell lineage preceding the differentiation of the guard cell. After the first asymmetric division that produces the stomatal initial, one daughter cell assumes a pavement cell identity and the other assumes a meristemoid identity. The meristemoid cell is capable of regenerating itself after each subsequent division, much as stem cells regenerate in animals. Meristemoids are stomatal initials because stomata do not originate from any other cell type. However, although meristemoids are necessary for stomatal formation, they are not sufficient. This is because stomatal formation requires that meristemoids subsequently assume the guard mother cell fate. The latter identity involves the commitment of the guard mother cell to divide symmetrically to produce two daughter cells, each of which assumes the same identity, that of a guard cell. In monocots, the stages leading to the choice of a stomatal cell fate are much more condensed than in dicots. Because the smaller product of the first asymmetric division in monocots develops into a guard mother cell without any intervening divisions, it is possible that cell fate may be partially committed before the asymmetric division.

Stomatal Development with Respect to Leaf Development

Monocot stomatal initials often form near the base of the leaf, and stomata mature basipetally (Sylvester et al., 1996). However, some monocots have linear arrays of stomatal precursors or developing stomata that are synchronized with respect to the stage of the cell cycle (Chin et al., 1995). These data suggest that the choice of cell fate depends on the stage of the cell cycle and that linear groups are patterned coordinately. Regardless of the degree of synchronization, once stomata form at a given position along the length of the monocot leaf, no new stomata develop during the subsequent maturation of this region.

In Arabidopsis and other dicots, most stomata are initiated and mature in a generally basipetal direction (Pyke et al., 1991), although additional meristemoids continue to form in intervening locations throughout the period of leaf growth. Thus, the competence to form stomatal initials extends over a wider area and for a longer developmental period in dicot leaves than it does in leaves of monocots.

If the placement of primary meristemoids in dicots is random, all protodermal cells should be competent to form stomata. However, in Arabidopsis leaves, meristemoids only form after trichomes do (Larkin et al., 1996). This could imply either that protodermal cells become competent to form stomatal meristemoids only after trichomes develop or that some inductive signal is transmitted only after this time. Regardless of the underlying mechanism, only a fraction of protodermal cells appears to form meristemoids.

It is possible that stomata that form later in developing Arabidopsis leaves arise mostly from satellite meristemoids. Smaller neighbor cells remain diploid after most other cells of the Arabidopsis leaf undergo endoreduplication (Melaragno et al., 1993). Presumably, meristemoid formation is more likely to occur in a diploid neighbor cell than in an endopolyploid pavement cell. If so, then the presence of diploid neighbor cells in an expanding leaf could function as a reservoir for the potential to produce stomatal initials.

Cell–Cell Communication in Stomatal Patterning

Intercellular signaling probably affects many aspects of stomatal development. Examples of possible cell–cell communication include (1) the inhibition of stomatal initial formation over veins (Smith and Watt, 1986; Sylvester et al., 1996); (2) the formation of satellite meristemoids positioned away from the stomate (Figure 3C); (3) the arrested development of meristemoids located too close to stomata or to each other (Sachs et al., 1993; Boetsch et al., 1995); (4) the orientation of corrective divisions so that meristemoids that form in contact with each other later become separated (Sachs et al., 1993); (5) the initiation of subsidiary cell formation in cells neighboring monocot stomatal initials (Figure 3A, diagrams ii and iii; Tomlinson, 1974); and (6) the induction of proliferative and oriented divisions several cells away from mature stomata (Galatis and Mitrakos, 1979; Sachs, 1994).

The classical lateral inhibition hypothesis of stomatal patterning invokes the presence of an inhibitory field around

developing stomata that prevents new stomata from forming in neighbor cells (Bünning, 1953; Korn, 1993, 1994). This hypothesis may explain the arrest of meristemoids that are located too close to stomata, but the nature of the inhibitory field, if it exists, needs to be determined. For example, it is equally possible that the examples of cell–cell communication cited above involve the binding of specific protein ligands and receptors rather than chemical (e.g., hormonal) or electrical fields.

GENETIC ANALYSIS OF STOMATAL PATTERNING

This discussion has established that stomatal spacing results from several different mechanisms, such as the polar placement of stomatal initials, the production and positioning of cells by the meristemoid or from adjacent cell lineages, and cell–cell communication at various levels. However, essentially nothing is known about the molecular or physiological nature of cell–cell signaling, the mechanism specifying the placement of asymmetric divisions, or the control of cell identity during stomatal development. Clearly, this area is ripe for molecular genetic analysis.

The study of the genetics of stomatal patterning is in its infancy compared with that of trichome development. Only a few mutations that disrupt stomatal patterning have been reported, and none of the corresponding genes has been cloned. Stomatal pattern mutants include the recessive mutations *too many mouths* (*tmm*), *four lips* (*flp*), and *R-558* in Arabidopsis (Yang and Sack, 1995; D. Berger and T. Altmann, personal communication) and the barley mutant *eceriferum-g*, which causes wax deficiencies and linear clusters of stomata on the leaf (Zeiger and Stebbins, 1972). Analysis of these mutant phenotypes is beginning to reveal the complexity of the mechanisms that regulate stomatal development.

TMM Controls Stomatal Initiation and Spacing

The *tmm* mutation causes a subset of stomata to form in large, frequently arc-shaped clusters in cotyledons and first-formed leaves (Yang and Sack, 1995). As shown in Figure 4C, the prohibition against stomata forming in contact in the wild type (Figure 4A) is violated by a defect in the *TMM* gene.

The *tmm* mutation also affects the formation of meristemoids. Compared with the wild type, *tmm* plants have more stomata in the cotyledon, rosette leaves, and abaxial epidermis of sepals. Other parts of *tmm* plants, such as the inflorescence stem, adaxial epidermis of sepals, and silique tips, entirely lack both stomata and meristemoids, although stomata are present in these regions in wild-type plants (Yang and Sack, 1995; M. Geisler, M. Yang, and F. Sack, unpublished data). The flower pedicel exhibits a dramatic gradient in the number of stomata produced, with none at the basal end and many more than normal at the apical (floral) end.

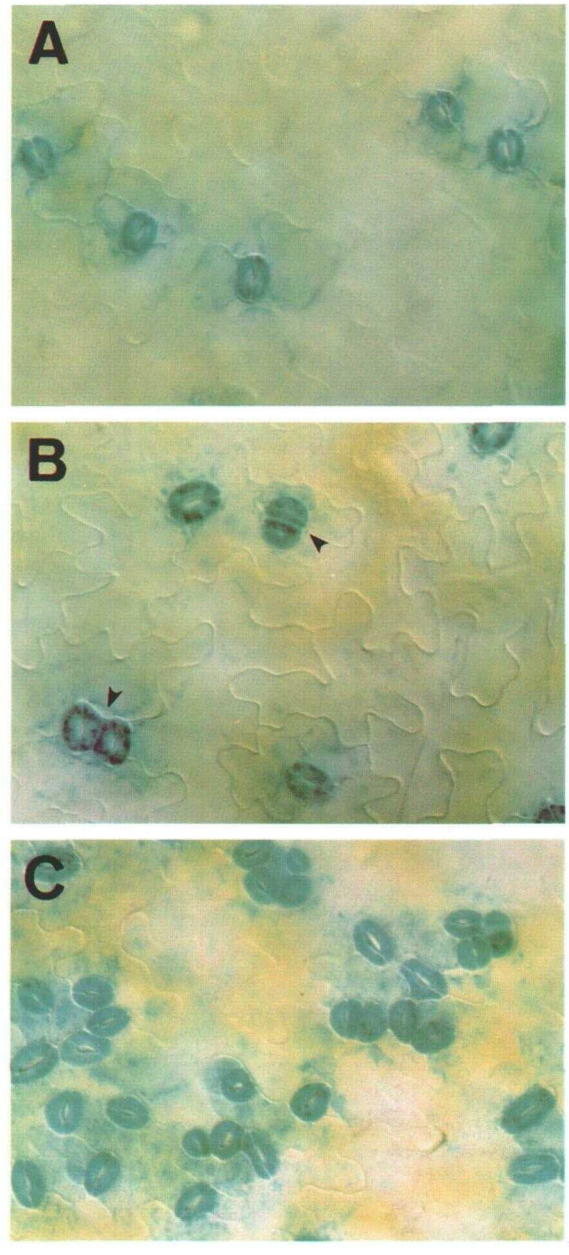

Figure 4. Stomata on Arabidopsis Wild-Type, *tmm*, and *flp* Leaves.

(A) Wild-type abaxial epidermis. Stomata do not form in contact with each other.

(B) *flp* epidermis. No increases in the number of meristemoids are apparent, but some stomata are twinned (arrowheads).

(C) *tmm* epidermis. Note the increased initiation of meristemoids; many stomata are found in clusters.

The guard cell–specific *KAT1* (*A. thaliana* potassium channel) promotor–GUS chimeric construct (courtesy of Rebecca Hirsch and Dr. Mike Sussman) was crossed into wild-type, *tmm*, or *flp* plants to visualize stomata after staining with 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid.

Thus, *TMM* also controls the entry of protodermal cells into the developmental program that leads to a stomatal lineage, and appears to have opposing effects on meristemoid initiation in different regions of the plant.

The developmental basis of the *tmm* phenotype is being analyzed through the use of sequential dental resin replicas of the same cells through time. Analysis to date reveals that at least some *tmm* clusters arise through the production of ectopic satellite meristemoids in the cotyledon. As shown in Figure 5C, the *tmm* mutation induces the overproduction of satellite meristemoids. Moreover, the polarity of some of the preceding asymmetric divisions seems to be altered such that meristemoids can form in contact with developing or mature stomata. Because the correct placement of satellite meristemoids could involve a form of lateral inhibition (see above), the determination of the molecular identity and function of the *TMM* gene may reveal basic mechanisms of plant cell patterning.

FLP May Regulate Guard Mother Cell Fate

The *flp* mutation results in the twinning of some stomata. It also causes unpaired guard cells to form individually or in contact with other guard cell pairs (Figure 4B; Yang and Sack, 1995; D. Berger and T. Altmann, personal communication). The three existing *flp* alleles exhibit a similar phenotype, suggesting that clustering in only a subset of stomata is a general characteristic of mutations at this locus. By contrast to *tmm*, *flp* does not have a significant quantitative effect on stomatal initiation, but like *tmm* mutants, *flp* plants exhibit a variable degree of stomatal clustering in different domains; cylindrical *flp* organs such as stems have fewer clusters than do dorsoventral organs such as leaves (M. Geisler and F. Sack, unpublished data).

Mutations at the *FLP* locus may disrupt the normal activity of the guard mother cell. Initial analysis of dental resin impressions suggests that the pattern of divisions leading to stomate formation is relatively normal until the guard mother cell stage in *flp* (Figure 5B). At this point, an aberrant symmetrical division may produce two adjacent cells that acquire the guard mother cell fate, that is, each of them subsequently divides symmetrically to form two guard cells. This suggests that *FLP* plays a role in establishing or maintaining guard mother cell identity or that it determines the number of divisions the guard mother cell will undergo once that identity has been established.

The *R-558* Gene Product Regulates Stomatal Density

The *R-558* mutation causes an increase in stomatal density throughout the Arabidopsis plant, although the extent of this increase varies in different organs (D. Berger and T. Altmann, personal communication). Stomatal clusters also are present, especially in cotyledons. Unlike *tmm*, these clusters never

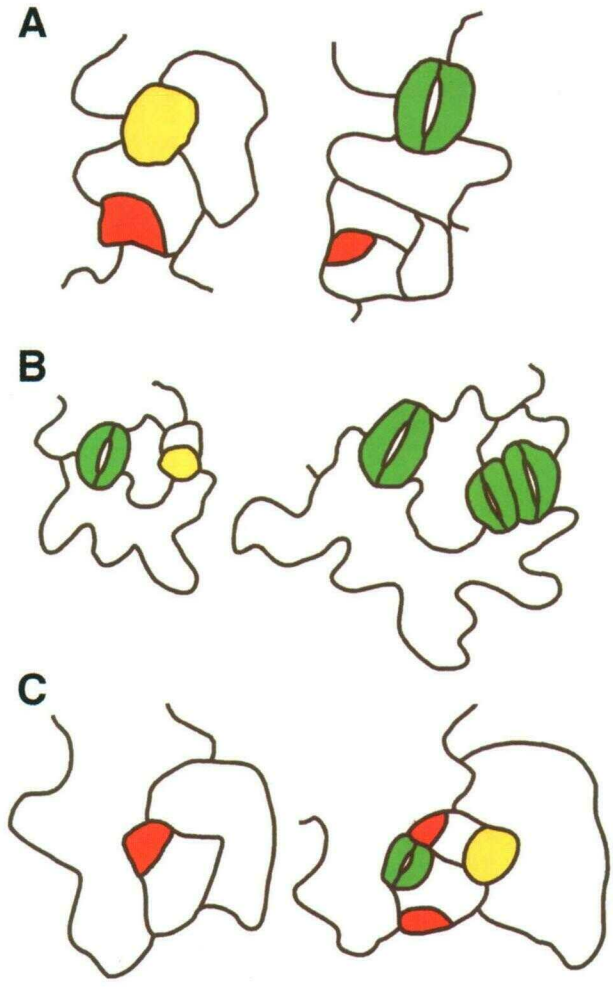

Figure 5. Normal and Aberrant Stomatal Patterning from Satellite Meristemoids in Wild-Type, *flp,* and *tmm* Arabidopsis Cotyledons.

Shown are tracings of dental resin impressions from the abaxial cotyledon epidermis. The second diagram in each panel represents the same region of the epidermis sampled 3 days after the first.

(A) Wild-type sequence showing normal patterning. Placement of the single satellite meristemoid away from the stomate and the production of new neighbor cells by the meristemoid separate the meristemoid from the stomate.

(B) *flp* sequence showing formation of twinned stomata. An aberrant guard mother cell (produced by a single satellite meristemoid) divides symmetrically across its short axis to produce two daughter cells, each of which differentiates into a stomate.

(C) *tmm* sequence showing initial stages of stomatal cluster formation. Three satellite meristemoids form, one of which is placed ectopically next to a stomate. Conversion of this meristemoid into a guard mother cell without undergoing asymmetric division(s) would result in two stomata, each produced by a different meristemoid, forming in contact with each other.

Red, meristemoid; yellow, guard mother cell; green, stomate.

contain more than four stomata and do not exhibit the densely packed and arc-shaped arrays that are characteristic of *tmm*. The developmental mechanisms underlying stomatal clustering and increased density in the *R-558* mutant are currently being analyzed.

COMPARISON OF STOMATAL AND TRICHOME PATTERNING

The patterning of Arabidopsis trichomes and stomata involves divergent mechanisms and genes, but some parallels exist. For example, current data suggest that both trichome precursor cells and primary meristemoids are selected randomly from fields of equally competent protodermal cells. The number of cells selected is under genetic control because many cells become pavement cells and because several mutations alter independently the number of stomata and trichomes. It is likely that the patterning of both trichomes and stomata results at least in part from communication between committed precursor cells or mature trichomes/stomata and surrounding cells.

The differences between stomatal and trichome patterning are also informative. Stomata originate from cell divisions that are unequal in both the size and fate of the two daughter cells, whereas no evidence exists in Arabidopsis for the creation of the trichome precursor cell by an asymmetric division. The production of cells by oriented divisions plays a key role in preventing clusters of mature stomata, whereas trichome spacing does not seem to depend on divisions of the trichome precursor cell or on specified cell lineages. Unlike primary meristemoids and trichome precursor cells, the placement of the satellite meristemoid usually is ordered, a spatial regulation that is critical to the generation of stomatal patterning from this latter class of initials.

So far as is known, trichome and stomatal patterning rely on different genes and exhibit qualitatively different phenotypes. Even though clustering of respective cell types occurs in mutants of both classes, *ttg* and *gl1* lack an aberrant stomatal phenotype, and *tmm* and *flp* lack a trichome phenotype (M. Geisler, M. Yang, and J. Nadeau, unpublished data). *ttg* and *gl1* eliminate trichomes from most of the plant, but *tmm* eliminates stomata only from specific domains while upregulating their number in other parts of the plant.

Progress in understanding these divergent genes and mechanisms requires the cloning and sequencing of key genes, including *TTG*, *TRY*, *TMM*, and *FLP*. It may also be possible to isolate new pattern mutants. This is especially true of stomatal patterning because the saturation screen for such mutants is not yet complete. Careful study of the same epidermis through time should reveal events involved in the patterning of trichomes and stomata in wild-type plants and in the mutants. It also will be important to investigate the factors that determine whether protodermal cells and some mature epidermal cells can become competent to form stomatal and trichome initials.

The isolation of mutants in Arabidopsis has accelerated progress in our understanding of epidermal cell fate specification and patterning. This progress has provided new ways of analyzing questions in a field of intense classical interest (stomatal patterning) and in one that had previously received much less attention (trichome patterning). Both systems show the promise of revealing how cell position and intercellular signaling establish different spacing patterns for different cell types in the same epidermis.

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NOTE ADDED IN PROOF

Recent results indicate that *TTG* is not an *R* homolog (A. Walker and J. Gray, personal communication). The identity of *TTG* will be reported soon.